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PRELIMINARY COMMUNICATION

INITIAL STUDIES ON THE CYTOTOXIC ACTION OF MAYTANSINE, A NOVEL ANSA MACROLIDE

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Maytansine (Fig. 1) is a new type of naturally occurring ansa macrolide, a class of compounds that includes the rifamycins and streptovaricins. It was originally isolated by Kupchan and coworkers from the East African shrub Maytenus serrata, and later from the wood and bark of Maytenus buchananii (1,2), and is the first ansa structure - an aromatic nucleus to which a macrocyclic aliphatic bridge is attached at two non-adjacent positions - to be isolated from a plant rather than a microorganism. Although previously described ansa macrolides are known for their inhibition of bacterial DNA-dependent RNA polymerase (3,4) and viral RNA-directed DNA polymerase (5), maytansine is the first compound of this class to show significant antitumor activity, as measured by prolongation of

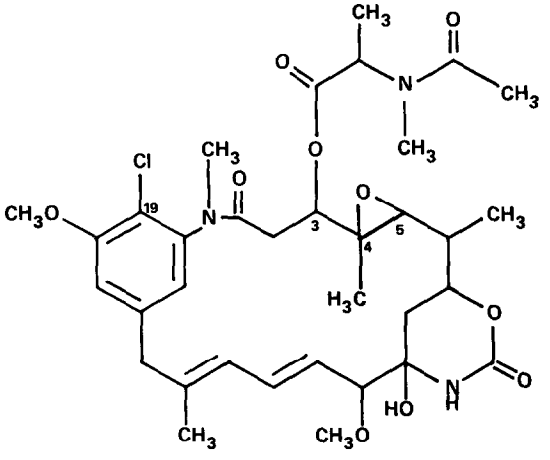

Figure 1. Structural formula of maytansine.

survival of mice bearing the P388 lymphocytic leukemia, the B16 melanocarcinoma, and the Lewis lung carcinoma (1,2). Initial studies on the mode of action of this compound in murine leukemia cells *in vitro* and *in vivo* are reported below.

The growth of L1210, L5178Y and P388 cells in suspension culture is suppressed by concentrations of maytansine in the nanomolar range, with the P388 murine leukemia line being the most sensitive (ED_{50} = 6 x 10^{-10}M at 48 hr). The ED_{50} for L1210 is 2 x 10^{-9}M and 1.5 x 10^{-9}M for L5178Y. At a low drug level (10^{-9}M), the inhibitory effect of maytansine could be reversed by washing and resuspending the cells in fresh medium after exposures to the drug as long as 24 hr; at maytansine levels of 10^{-8}M or higher, growth inhibition could not be reversed by washing. Histological examination of L1210 cells fixed in alcohol and stained with Giemsa after a 24-hr exposure to 10^{-8}M maytansine showed 30%

Table 1. EFFECT OF MAYTANSINE ON THYMIDINE, URIDINE AND LEUCINE INCORPORATION BY MURINE LEUKEMIA CELLS

	Incorporation (cpm x 10^{-3}/5 x 10^{5} cells/30 min)		
Cell type	Thymidine	Uridine	Leucine
L1210 Control	5.5 ± 1.6	4.9 ± 1.4	2.2 ± 0.7
Maytansine	2.9 ± 0.6 (53)*	6.6 ± 2.8 (135)	0.9 ± 0.2 (41)
L5178Y Control	6.6 ± 0.7	4.3 ± 1.5	1.1 ± 0.3
Maytansine	1.8 ± 0.3 (27)	4.6 ± 0.9 (107)	1.2 ± 0.3 (109)
P388 Control	14.0 ± 2.2	11.5 ± 0.5	2.3 ± 0.4
Maytansine	2.0 ± 0.3 (14)	5.3 ± 0.8 (46)	1.1 ± 0.1 (48)

Cells in log phase growing in a 5 ml volume of RPMI 1630 with 10% fetal calf serum, or Fischer's medium with 10% horse serum in the case of P388 cells, were exposed to maytansine, 10^{-7}M, for 12 hr, followed by a 30-min pulse with one of the following labeled precursors: (1) 10 µCi of methyl-^{3}H-thymidine (1.9 Ci/m-mole; Schwarz/Mann); (2) 10 µCi of 5-^{3}H-uridine (2 Ci/m-mole; Schwarz/Mann), or (3) 10 µCi of 4,5-^{3}H-L-leucine (6 Ci/m-mole; Schwarz/Mann). Cells were washed, precipitated with 5% perchloric acid and collected on Whatman glass fibre filters (GR/C). Radioactivity was determined by means of a Beckman LS-230 liquid scintillation counter. Values represent the mean ± S.E. of 4 determinations. *Values in ( ) represent % of control.

with mitotic figures, as compared to 3% for control cells.

Table 1 shows the effect of 12 hr of exposure to maytansine, 10^{-7}M, on the incorporation of methyl-^{3}H-thymidine into DNA, 5-^{3}H-uridine into RNA, and 4,5-^{3}H-leucine into protein, in the three cell lines utilized in this study. Of the three macromolecular synthetic processes examined, DNA synthesis was inhibited to the greatest extent. Although RNA synthesis was inhibited to a lesser degree, the sensitivity of RNA polymerase to this agent was determined in view of the unusual sensitivity of this enzyme to inhibition by other agents of the ansa macrolide class. When assayed by the method of Burgess and Travers (6), E. coli RNA polymerase was not inhibited at maytansine levels as high as 10^{-4}M.

Table 2. EFFECT OF MAYTANSINE AND VINCRISTINE ON SURVIVAL TIME OF MALE CDF_1 MICE BEARING VINCRISTINE-SENSITIVE AND VINCRISTINE-RESISTANT P388 LEUKEMIA

Compound	Dose range tested (mg/kg)	Optimal daily dose (mg/kg)	Tumor	Mean survival at optimal dose (days ± S.E.)
Controls			P388*	10.8 ± 0.3
Maytansine	0.025 - 0.100	0.025	P388	21.1 ± 1.4
Vincristine	0.25 - 0.50	0.25	P388	24.3 ± 1.3
Controls			P388/VCR†	10.1 ± 0.3
Maytansine	0.025 - 0.100	0.025	P388/VCR	10.2 ± 0.3
Vincristine	0.25 - 0.50	0.25	P388/VCR	9.6 ± 0.2

Mice received 10^{6} tumor cells i.p. Drug treatment was started 24 hr later by the i.p. route, and was continued once daily for 10 days. Each treatment group consisted of 10 mice. *Vincristine-sensitive; †Vincristine-resistant.

Because of the elevated mitotic index seen after exposure of cells to maytansine in suspension culture, both vincristine-sensitive and vincristine-resistant cell lines were used to examine antitumor activity of maytansine in vivo. Maytansine, administered intraperitoneally, was effective at extremely low doses (25 - 50 μg/kg) in prolonging the survival of mice bearing the vincristine-sensitive P388 leukemia in ascites form (Table 2); these doses are some 10-

fold lower than the effective therapeutic levels for the reference compound, vincristine. Of particular interest for future studies on the mode of action of maytansine was the lack of antitumor activity of the drug in the vincristine-resistant line of the P388 tumor (Table 2).

These initial studies indicate that maytansine, the first ansa macrolide found to show significant antitumor activity, is active in vitro and in vivo at extremely low dose-levels, with the biosynthetic process most sensitive to the drug being DNA synthesis. Whether the observed inhibition is a direct effect on one of the steps of the DNA biosynthetic process, or a secondary effect due to drug-induced arrest of cells at a specific phase of mitosis, is yet to be established; the cross-resistance with vincristine indicates, however, that the second possibility merits further study.

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